ION CURRENTS THROUGH PORES

The Roles of Diffusion and External Access Steps in Determining the Currents through Narrow Pores

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ABSTRACT External access steps, which may include restricted aqueous diffusion, are introduced into a kinetic model for ion transport through narrow pores. The conductance-concentration relation and the concentration dependence of the biionic permeability are calculated using two alternative assumptions: (*a*) access to the mouth of the pore is allowed only when no ion is within the lumen or at either mouth; (*b*) ions remain at the mouth only very transiently. With either assumption the concentration dependence of the fluxes is the same as in previous treatments in which all steps in access were lumped into a single process. Also as before, the biionic permeability ratio is independent of concentration so long as the lumen is never doubly occupied. For narrow pores, such as those formed by gramicidin A, the slowest external portion of the access process must occur close to the pore's mouth, and thus the region an ion must occupy to gain access is small. As a consequence, the probability of finding an ion within this region is also small. On this basis, it is argued that the second assumption is appropriate for these pores. The kinetic equations that result are identical to those used by Urban, B., S. B. Hladky, and D. A. Haydon (1980, *Biochim. Biophys. Acta.* 602:331–354).

INTRODUCTION

The ion currents through single channels formed by gramicidin A have been measured on numerous occasions. These data have been used to infer that the channels are narrow pores filled with water (Hladky and Haydon, 1972) and to characterize the process by which ions pass through the channels. It has been concluded that significant binding of ions to the pore occurs for concentrations below 0.1 M (see Neher et al., 1978; Urban et al., 1980; Dani and Levitt, 1981; Finkelstein and Andersen, 1981), that movement of ions into the pore is rate limiting at low concentrations (Hladky and Haydon, 1972; Urban et al., 1980; Andersen, 1983*a*), and that two ions can simultaneously be bound to the pore at high concentrations (Hladky, 1972; Myers and Haydon, 1972; Neher et al., 1978; Urban et al., 1980; Finkelstein and Andersen, 1981). Recently, Andersen (1983*a*, *b*) proposed that aqueous diffusion is rate limiting and that failure to consider this limitation seriously compromises previous attempts to interpret the kinetic data. It is therefore necessary to consider in some detail the kinetic consequences of slow, external access steps. These consequences depend critically upon how this slow step is affected by ion binding within the lumen of the pore.

THE DIFFUSION EQUATIONS ARE INADEQUATE TO MODEL ACCESS TO NARROW PORES

Explicit calculation of the effects of diffusion or slow external access steps on the currents through narrow pores is not straightforward. In the standard calculation for wider pores (Ferry, 1936), a solute is presumed to have arrived at the pore only when the distance between its center and the center of the mouth is less than the difference in their radii, i.e., when the solute is obviously entering the pore and is clear of the pore's rim. This approach has been used, with reasonable success, to describe the access of large, fully hydrated molecules into wide pores such as those in the walls of capillaries in the cardiovascular system (see Landis and Pappenheimer, 1963). For these pores and solutes, interactions with the walls of the pore (other than steric repulsion and hydrodynamic drag) are incidental to the transport process; the differences in radii are usually many angstroms, i.e., long compared with a typical jump length for a particle diffusing in free solution, and the solutes do not interact with each other during transport. Ions and narrow pores like gramicidin are quite different in that the difference in radii between the ions and the pore is much smaller than a water molecule; partial dehydration must occur for ions to enter the pore; the ions interact specifically with the pore structure, and there is definite repulsion between ions associated with the pore. Thus, the success of the traditional theory in dealing with large pores has little bearing on its relevance for narrow pores.

It is important to realize that severe difficulties arise in any attempt to use the diffusion equations to predict the rate at which ions can enter a narrow pore. For a 2-Å-radius pore like gramicidin, the center of the ion must pass through a disk with radius ~0.3 Å. For such a small target, the standard diffusion equation for radial symmetry,

$$J = 2\pi r^2 D \frac{dc}{dr}, \tag{1}$$

where J is the flux through the pore and D is the diffusion constant, predicts that 90% of the concentration difference driving the diffusion falls within 3 Å of the center of the target and hence the center of the mouth of the pore. There are several consequences of this small distance. First, it is not clear that concentration differences between points separated by less than the size of a water molecule can be calculated using diffusion equations that treat the solvent as a continuum and ion movement as an infinite series of infinitesimal jumps. Second, any ion within the region of altered concentration will be in contact with the pore structure and partially dehydrated, which may affect the concentration at equilibrium and the rates of ion movements. Finally, and for the present purposes most significantly, only one ion can possibly be in this region at a time, and if present it will be close to and hence will interact strongly with any ion within that end of the pore. Thus, any acceptable calculation for a target this small must allow for a difference between the equilibrium concentration at the mouth of the pore and the concentration in bulk solution, it must deal with individual ions, and it must take into account the repulsion between ions in the access region and in the lumen. The standard diffusion equations do none of these.

THE USE OF EXTERNAL VIRTUAL SITES TO MODEL THE ACCESS PROCESS

The difficulties encountered in the use of the diffusion equations can be minimized if, for a narrow pore like gramicidin, changes in the concentration within the small volume just outside the mouth of the pore are modeled as changes in the occupancy of an extra superficial site. The external access process is then assigned an explicit rate constant. Entry into the lumen is represented by the transition between the external site and an internal site near the same end, and transfer through the pore is represented by movement between the two internal sites. It should be noted that there is no assurance that any of these transitions correspond to single molecular jumps.

The formal equations describing the transport scheme outlined above are those of a four-site pore. The general solution of the model equations for the net flux is still quite complex (there are 2^4 occupancy states and many more rate constants) and it will not be attempted here. Eisenman and his colleagues (1980, 1982) have solved the equations for two special cases—outer sites at equilibrium with the adjacent aqueous phases (their 3B4S" model; Eisenman et al., 1982), and outer and inner sites at equilibrium with each other (3B3S'; see Eisenman et al., 1980). However, neither of these solutions is appropriate to deal with rate-limiting external access and weak external binding. The first condition alone precludes the 3B4S" model. With both conditions, the 3B4S' model requires impossibly large rate constants for exchange between sites. Fortunately, there are other special cases with comparatively simple solutions which illustrate the important points.

Single Occupancy of the Pore

For symmetrical pores that are never more than singly occupied (all four sites), the flux for a small applied potential may be written down immediately as a special case of Läuger's (1973) equations for singly occupied pores:

$$J = \frac{-\dfrac{k_1 k_2}{k_{-1} k_{-2}}\, l c \Delta\phi}{(1 + K_p c)\left[1 + \dfrac{2l(k_{-2} + k_1)}{k_{-1} k_{-2}}\right]}, \tag{2}$$

where J is the flux, $\Delta\phi = ze\Delta V/kT$, ze is charge on the permeant ion, ΔV is the applied potential, $kT/e = 25$ mV at 20°c, K_p is the equilibrium binding constant for ions to the pore, and the rate constants represent the following ion movements: k_2, 0 (= solution) to 1 and 5 (= solution) to 4; k_{-2}, 1 to 0 and 4 to 5; k_1, 1 to 2 and 4 to 3; k_{-1}, 2 to 1 and 3 to 4; and l, 2 to 3 and 3 to 2. The equilibrium binding constant is related to the rate constants by

$$K_p = \frac{2k_2}{k_{-2}}\left(1 + \frac{k_1}{k_{-1}}\right). \tag{3}$$

Thus, whenever ion repulsion limits occupancy to only one ion, the flux increases with concentration up to a limit, and the concentration for half-maximal flux is just $1/K_p$. It also follows directly from Läuger's equations that the strict biionic permeability ratio is independent of concentration and equal to the ratio of the conductances at low concentrations, provided this ratio is determined at the same potential. It is, of course, unlikely that an ion bound at one end would prevent an ion from binding to an external site at the other end. However, if the external sites represent the region of the aqueous phase where the concentration deviates appreciably from its equilibrium value, e.g., the volume of a hemisphere of radius 2 Å, which is ~17 Å^3, these sites will rarely be occupied even in the absence of such repulsion, and Eq. 2 will be applicable. This statement is justified in more detail in the Appendix, where Eq. 2 is compared with an alternative approximation derived by Andersen (1983*b*).

Transient Binding to External Sites

There is now general agreement that gramicidin pores can be occupied by more than one ion at a time. A solution to the kinetic equations for narrow pores can be obtained without the arbitrary restriction to single occupancy if it is assumed that binding to the external sites is weak and transient. Weak and transient binding follows necessarily if the external sites represent the regions of the aqueous phases that lie just outside the mouths of the pore. Thus, the rate constant for arrival of ions at one of these sites from the bulk of the aqueous phase can then be estimated from the radial diffusion equation. For $D = 2.10^{-5}$ cm, $\mathcal{A}$ = Avogadro's number, and a radius, $r > 0.3$ Å, the rate constant is $k_2 = 2.10^{-3}\ \pi r D \mathcal{A} > 2.3 \times 10^8\ \mathrm{M}^{-1}\ \mathrm{s}^{-1}$. Similarly, the probability that this region holds an ion when the concentration is 1 M can be estimated as the ratio of the volume of the access region to the volume per ion in solution. This is ~0.01. Thus, the "binding constant" to the external site is of the order of $10^{-2}\ \mathrm{M}^{-1}$, which represents weak binding, and the rate constant for ions leaving this site into the aqueous solution becomes $k_{-2} > 2 \times 10^{10}\ \mathrm{s}^{-1}$, which implies that the binding is very transient. Entry into the lumen (k_1) may be as fast (Andersen's [1983*a*] experimental results for gramicidin imply that at high potentials $k_1 > k_{-2}$), but no other step in transport through a narrow pore is likely to approach this rate since transfer through the lumen (l) involves movement over a much longer distance, while exit from the lumen to the virtual site (k_{-1}) must be much slower than entry (k_1) if there is any detectable ion binding. These conditions still allow the external access step (k_2) to be rate limiting.

If, as just suggested, ions leave the external sites much more rapidly than any other movements in the model, it is permissible to assume that whenever an ion enters an external site, it will proceed either to the aqueous phase or to an internal site before any other transitions have time to occur. With this assumption, it follows by standard methods that the conductance is given by

$$G = \frac{(ze)^2}{kT} \frac{\frac{AK}{B}}{\left(1 + \frac{2A}{B} + \frac{AD}{BE}\right)\left(1 + \frac{2K}{D + B}\right)}, \tag{4}$$

where the composite constants describe the following processes:

$A = (k_2ck_1)/k_{-2} + k_1)$, access to an inner site when the pore is empty;

$B = (k_{-2}k_{-1})/(k_{-2} + k_1)$, exit from an inner site when the other is empty;

$D = (d_2cd_1)/(d_{-2} + d_1)$, access to an inner site when the other is occupied.;

$E = (d_{-2}d_{-1})/(d_{-2} + d_1)$, exit from an inner site when the other is occupied; and

$K = l$, transfer between the internal sites.

Eq. 4 is identical to the conductance equation for the two-site model (Urban and Hladky, 1979) and this identity extends to all other flux expressions. In other words, in this approximation, the four-site model amounts to an interpretation of the two-site model in which the access and exit processes are each broken down into internal and external stages.

CONCLUSION

For narrow pores, ions must diffuse up to the mouth and be partially dehydrated before they can enter the pore lumen. Hladky and Haydon (1972) and Urban et al. (1980) demonstrated for gramicidin that some part of access to the pore is rate limiting at low concentrations and Andersen (1983*a*, *b*) has extended these results to show that at high potentials the limiting step occurs outside the lumen. Therefore, it is clear that all parts of the access process must be taken into account in producing a kinetic model for the ion fluxes. However, for narrow pores like those formed by gramicidin, the rate-limiting step must occur so close to the mouth of the pore that, in effect, only those ions that pass through this step while the near end of the pore is empty are able to gain access to the pore. It then becomes a matter of taste whether the access process is broken into internal and external components as here or treated as a composite process as in the approach used by Urban et al. (1980). Regardless, the conductance-activity relation then closely reflects the binding of ions to the pore and the permeability ratios are independent of concentration unless more than one ion can enter the pore.

APPENDIX

Andersen (1983*b*) has derived a flux equation for two-site pores with extra, external access steps at the ends. He has explicitly restricted the theory to single occupancy of the pore interior and has implicitly assumed that external binding is negligible since in his equations, ions near the mouth of the pore never get in the way of those coming out. His equation can be written in the present notation (in a slightly extended form) as

$$J = \frac{-\frac{k_1k_2}{k_{-1}k_{-2}} lc\Delta\phi}{1 + \frac{2l(k_{-2} + k_1)}{k_{-2}k_{-1}} + \left(1 + \frac{2l}{k_{-1}}\right)\left(\frac{2k_1k_2c}{k_{-1}k_{-2}}\right)}. \tag{A1}$$

For comparison, when there is negligible external binding, Eq. 2 becomes

$$J = \frac{-\frac{k_1k_2}{k_{-1}k_{-2}} lc\Delta\phi}{\left(1 + \frac{2k_1k_2c}{k_{-1}k_{-2}}\right)\left(1 + \frac{2l(k_{-2} + k_1)}{k_{-1}k_{-2}}\right)}. \tag{A2}$$

In these equations, the symbols have the same physical meaning. For instance, in both equations k_2 represents movement from the bulk phase to the mouth of the pore, a process that may be diffusion limited. Since these equations are different, they cannot both be a correct description of diffusion-limited access to a narrow pore. In fact, both are approximations.

For small applied potentials and hence a small difference in the probabilities of finding an ion near the two mouths of the pore, both calculations in effect make use of the following relation for transfer through the lumen since both assume the same model for the pore interior:

$$J = -\frac{\dfrac{k_1 l k_2 c}{k_{-1}k_{-2}}\Delta\phi + \dfrac{k_1 l}{k_{-1}}\Delta X}{\left(1 + \dfrac{2l}{k_{-1}}\right)\left(1 + \dfrac{2k_1 k_2 c}{k_{-1}k_{-2}}\right)}, \tag{A3}$$

where

$$\frac{k_2 c}{k_{-2}} = X''_{1:00} + X'_{1:00}, \tag{A4}$$

$$\Delta X = X''_{1:00} - X'_{1:00}, \tag{A5}$$

when $X'_{1:00}$ is the conditional probability that an ion is located just outside the pore on the left given that the interior is empty, and $X''_{1:00}$ is the corresponding conditional probability on the right.

Similarly, both calculations make use of the relation between the net flux up to the mouth of the pore and the average probability of finding an ion at the mouth. On the left this can be written,

$$J = k_2 c - k_{-2}\left(X'_{1:00}X_{00} + X'_{1:10}X_{10} + X'_{1:01}X_{01}\right), \tag{A6}$$

where the quantity in parentheses is the average probability, X_{00}, X_{10}, and X_{01} are the probabilities that the pore interior is empty, occupied on the left, or occupied on the right, respectively, and $X'_{1:00}$, $X'_{1:10}$, and $X'_{1:01}$ are the conditional probabilities that an ion is located just outside the pore on the left, given that the pore interior is empty, occupied on the left, or occupied on the right. In Eq. A6, the probability that ions are located at both mouths simultaneously has been assumed to be negligible.

Eq. A2 now follows by elementary algebra if there is no net flux to the mouth of the pore when the pore interior is occupied, i.e., when

$$k_2 c(X_{01} + X_{10}) - k_{-2}(X'_{1:10}X_{10} + X'_{1:01}X_{01}) = 0 \tag{A7}$$

and

$$J = k_2 c X_{00} - k_{-2}X'_{1:00}X_{00}. \tag{A8}$$

By contrast, Eq. A1 is based on the assumption that the average probability that an ion is at the mouth of the pore is the same as the probability that an ion is at the mouth when it is possible to enter the pore, i.e., that

$$X'_{1:00} = X'_{1:00}X_{00} + X'_{1:10}X_{10} + X'_{1:01}X_{01}, \tag{A9}$$

which leads to

$$J = k_2 c - K_{-2}X'_{1:00}. \tag{A10}$$

This equation, its parallel on the opposite side, Eq. A3, and Eq. A5 lead directly to Eq. A1.

Neither Eq. A8 nor A10 is exact. However, for narrow pores with only two genuine binding sites, Eq. A8 is likely to be much nearer the mark. There are two reasons. First, ion repulsion will effectively prohibit external access when the near internal site is occupied. Second, for weak, transient external binding, the conditional probabilities are not equal even in the absence of long range repulsion between ions on internal and external sites. The origin of the difference between $X'_{1:00}$ and either $X'_{1:10}$ or $X'_{1:01}$ can be understood by considering a pore with rapid internal transfer, i.e., $l >> k_{-1}$, and the fate of ions that approach it. If the pore is occupied, these will return to the solution with rate constant k_{-2}, unless the pore becomes vacant first, which will occur at a rate of the order of k_{-1}. If, as is likely, departure from the mouth of the pore is much faster than dissociation from the sites within the lumen, i.e., $k_2, >> k_{-1}$, the concentration (i.e., the frequency of finding an ion averaged over many such occurrences) at the mouth comes to equilibrium with the bulk phase within a time of $4K_{-2}^{-1}$, which is much shorter than the residence time of an ion within the pore (of the order of $1/k_{-1}$ s). Thus, most ions that arrive while the pore interior is occupied also leave and do not contribute to the flux. This is equivalent to saying that $X_{1:10} \simeq X_{1:01} \simeq k_2 c/k_{-2}$, which when inserted into Eq. A6 yields A8, which in turn leads to A2. Quantitatively, if internal transfer is fast and only internal transfer depends on the applied potential, then for small applied potentials

$$X'_{1:00} \simeq \frac{k_2 c}{k_{-2}}\left[1 - \frac{k_1\Delta\phi}{2(k_{-2} + d_1)}\right] \tag{A11}$$

and

$$X'_{1:10} = X'_{1:01} \simeq \frac{k_2 c}{k_{-2}}\left[1 - \frac{k_{-1}\Delta\phi}{2(2k_{-2} + k_{-1})}\right]. \tag{A12}$$

After inserting Eqs. A11 and A12 into A6, the terms in X_{10}, and X_{01} are negligible for

$$\frac{2k_1 k_2 c}{k_1 k_{-2}}\left[\frac{k_{-1}}{2(2k_{-2} + k_{-1})}\right] << \frac{k_1}{2(k_{-2} + k_1)}; \tag{A13}$$

i.e., for

$$\frac{2k_2 c}{k_{-2}}\frac{(k_1 + k_{-2})}{(2k_{-2} + k_{-1})} << 1, \tag{A14}$$

which for transient external binding is roughly

$$\frac{2k_2 c}{k_{-2}} << 1. \tag{A15}$$

As argued in the main text, this condition will be satisfied for diffusion-limited access to a narrow single-occupancy pore, and thus for these conditions, Eq. A2 is the appropriate approximation.

Received for publication 19 August 1983 and in final form 15 February 1984.

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